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The intramandibular gland of *Aneuretus simoni* (Formicidae, Aneuretinae)

Johan Billen and Sebastiaan Verbesselt

Zoological Institute, University of Leuven, Naamsestraat 59, box 2466, B-3000 Leuven (Belgium)

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Contact address:

Johan Billen, KU Leuven, Zoological Institute, Naamsestraat 59, box 2466, B-3000 Leuven, Belgium
Tel : (32) 16 323975
Fax : (32) 16 324575
E-mail johan.billen@bio.kuleuven.be

Abstract

Aneuretus simoni workers have a conspicuous intramandibular gland, formed by round to polygonal class-3 secretory cells with a diameter around 16-17 μm and their accompanying duct cells. Both in minor and major workers, approx. 8 cells open through the proximal upper surface of the mandible, approx. 12 cells open through the proximal lower surface. The secretory cells are characterized by a well developed granular endoplasmic reticulum and Golgi apparatus. The cytoplasmic organization indicates the secretion has a proteinaceous nature, and therefore probably does not play a pheromonal role. The precise function, however, is not yet known.

Introduction

Aneuretus simoni Emery, 1893 is endemic to Sri Lanka, and represents the only living representative of the subfamily Aneuretinae. The small polydomous colonies contain one or sometimes more dealate queens, up to 3 major and an average of 65 minor workers. The major workers are not involved in defence but rather play a role in food storage, whereas the minors are in charge of brood care, defence and foraging (Jayasuriya & Traniello 1985).

Because of its unique phylogenetic status together with its limited geographical distribution in southern Sri Lanka only, *A. simoni* since 1996 has been included in the IUCN Red List as 'critically endangered'. This was adjusted to 'endangered' (Fellowes & Brühl 2009), as more sites have been recently reported where the ants have been found (Dias et al. 2011, 2013; Dias & Ruchirani 2014). In all ant phylogenetic studies (e.g. Brown 1954; Wilson et al. 1956; Taylor 1978; Brady et al. 2006; Ward 2007; Moreau & Bell 2013), the Aneuretinae are considered the sister group to the Dolichoderinae, based on fossil, anatomical as well as genetic grounds.

From the viewpoint of exocrinology, the close relationship between Aneuretinae and Dolichoderinae is supported by the presence of Pavan's gland, that is only found in these two subfamilies. We undertook a study of the exocrine system of *A. simoni*, and here report on the morphology and ultrastructure of their conspicuous intramandibular gland.

Material and methods

During a visit to Gilimale Forest in southern Sri Lanka in December 1986, we collected a nest fragment of *Aneuretus simoni* in a decomposing fallen twig. This sample contained 16 minor and 4 major workers (note that literature data mention a maximum number of only 2 [Wilson et al. 1956] resp. 3 major workers per colony [Jayasuriya & Traniello 1985]). The anterior head parts of 13 minor and 2 major workers were fixed in 2% glutaraldehyde, buffered at pH 7.3 with 50 mM Na-cacodylate and 150 mM saccharose. Postfixation was carried out in 2% osmium tetroxide in the same buffer. After dehydration in a graded acetone series, tissues

were embedded in Araldite and sectioned with a Leica EM UC6 ultramicrotome. Semithin 1 µm sections were stained with methylene blue and thionin and viewed in an Olympus BX-51 microscope; double stained 70 nm thin sections were examined in a Zeiss EM900 electron microscope. The heads of the remaining 3 minor and 2 major workers were mounted on stubs, coated with gold, and examined in a JEOL JSM-6360 scanning microscope.

Results

Semithin sections through the mandibles of both minor and major workers revealed the presence of a prominent intramandibular gland (Fig. 1A,B). In both castes, the gland consists of approx. 20 rounded to polygonal class-3 cells according to the classification of Noirot and Quennedey (1974). The secretory cells have a diameter of 16.6 ± 2.7 µm in minor (n = 40 cells measured) and 15.6 ± 2.7 µm in major workers (n = 7 cells). Each cell is connected to its accompanying duct cell, thus forming a bicellular unit. Approx. 8 of these units open through the upper surface of the mandible, whereas the other 12 open through the lower surface. Scanning microscopy shows the duct openings in the proximal half of the mandibular surface as isolated pores with a diameter around 0.25 µm (Fig. 1C,D). Counts of the number of pores are line with the number of secretory cells as estimated from the serial semithin sections.

Electron microscopy shows that both the upper and lower cells have a highly vacuolar cytoplasm and a rounded nucleus with a diameter around 5 µm (Fig. 2A). At higher magnification, the vacuolar organization corresponds with a well developed granular endoplasmic reticulum, while the cytoplasma also contains a well developed Golgi apparatus but rather few mitochondria (Fig. 2B,D). Each secretory cell has a curved end apparatus with clear microvilli, as can be understood from the 2-3 times it is hit on a section (Fig. 2A,C). In the vicinity of the end apparatus, lamellar inclusions with a diameter of 1 µm can be found (Fig. 2C). The duct cells have a very reduced cytoplasm, and are mainly made up by the sclerotized ductule, through which the secretory products are transported to the outside. The ductule wall has a thickness around 0.1 µm and an internal diameter around 0.25-0.5 µm (Fig. 2B,D).

Discussion

Social insects in general, and ants in particular, are known for their extremely diversified exocrine system. The number of known glands for all social insects together is currently 149, of which 84 are reported for ants (Billen & Šobotník 2015). Intramandibular glands formed by class-3 secretory cells are a fairly common exocrine structure in ants, where they were first described by Schoeters and Billen (1994), and later also found by Grasso et al. (2004), Amaral and Caetano (2006) and Martins and Serrão (2011). They had also been described in stingless bees (Costa-Leonardo et al. 1978) and recently also in wasps (Penagos-Arévalo et al. 2015). Besides the class-3 intramandibular gland cells, Costa-Leonardo et al. (1978) in some meliponine bees also reported on the occurrence of an intramandibular gland made up of class-1 cells. We also found such epithelial intramandibular glands with class-1 cells in *Strumigenys (Pyramica) membranifera* (Billen & Espadaler 2002) and in *Protanilla wallacei* (Billen et al. 2013). The structural variation of intramandibular glands is further illustrated by the occurrence in *Tatuidris tatusia* of class-3 secretory cells, but here the cells do not open through isolated pores on the mandibular surface, but through a single pore plate at the ventral proximal side of each mandible (Billen & Delsinne 2014).

The function of intramandibular glands remains rather unknown so far. The only clearly illustrated function is that of substrate marking in order to recruit nestmates as shown by Roux et al. (2010) for the class-3 intramandibular gland in the weaver ant *Oecophylla longinoda*. Such pheromonal function goes along with the elaboration of small non-proteinaceous molecules, which in turn corresponds with the presence of a well-developed smooth endoplasmic reticulum, as usually found in intramandibular gland cells (Schoeters & Billen 1994). Our finding of a clear granular endoplasmic reticulum in *Aneuretus simoni*, however, supposes the production of a proteinaceous and hence non-pheromonal secretion. Glycoprotein synthesis was already reported for the intramandibular glands of leaf-cutting ants (Amaral & Caetano 2006), while also Martins et al. (2015) found clear protein presence in *Atta laevigata* workers. The real function of such secretion, however, remains unknown. The presence of lamellar bodies is commonly found in exocrine gland (Billen 1991), and may be regarded as lipidic secretion (Boudreau et al. 1983). A peculiarity of the intramandibular gland of

A. simoni is that the secretory cells discharge their secretion both through the upper and lower side of the mandible, whereas in most ants they open through the upper surface (Schoeters & Billen 1994). The opening at both sides may be linked with a more pronounced distribution of the glandular secretion. The similar development of the intramandibular gland in both minor and major workers indicates the gland does not have a caste-specific function, although careful observations of eventual peculiar mandibular movements and use of mandibular extracts will be needed to clarify the function of this gland in these ants.

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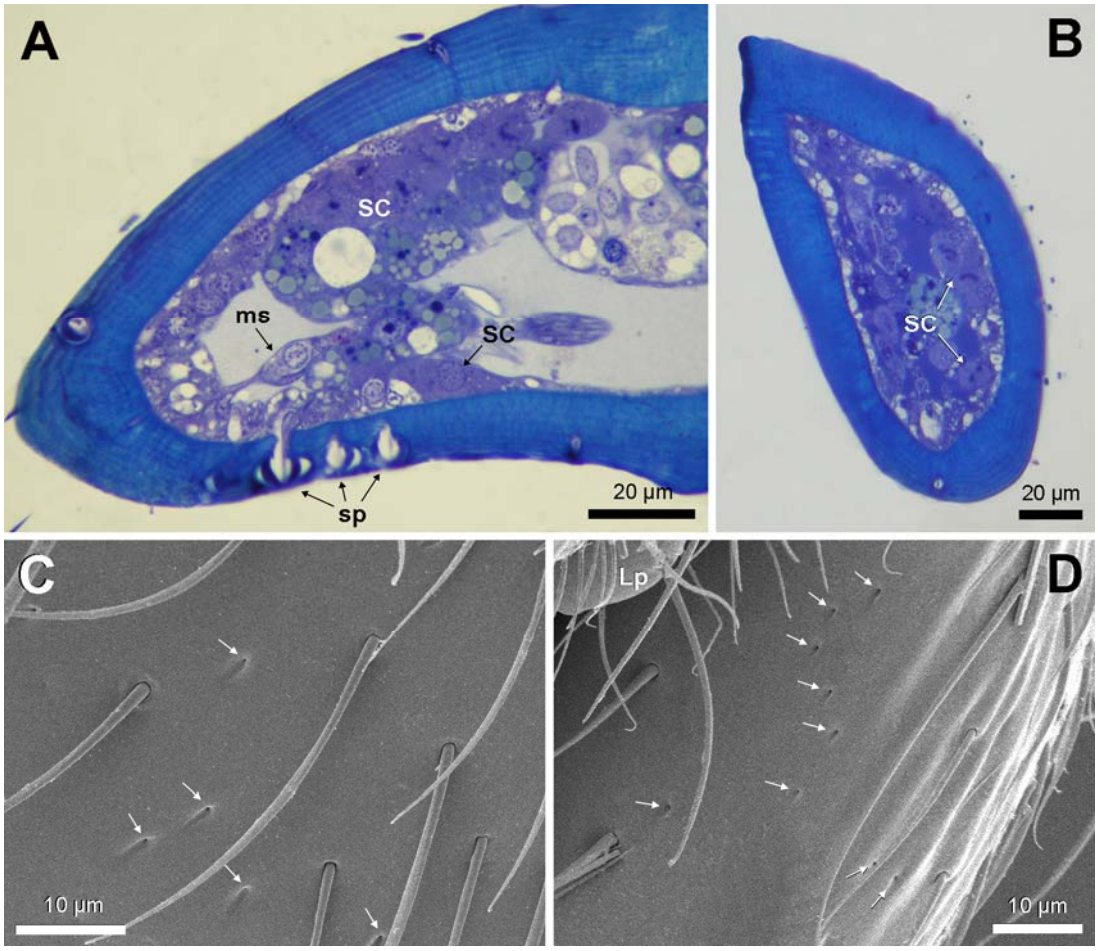
202 **Figure legends**



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204 Fig. 1 - **A**. Longitudinal section through mandible of minor worker, showing secretory
205 cells (SC) underneath both upper and lower cuticle. ms: mandibular sensillae,
206 sp: sensillar pores. **B**. Cross section through mandible of major worker with
207 secretory cells (SC). **C**,**D**. scanning micrographs of upper (C) and lower (D) part
208 of proximal mandibular surface of minor worker, showing scattered openings of
209 intramandibular gland ducts.

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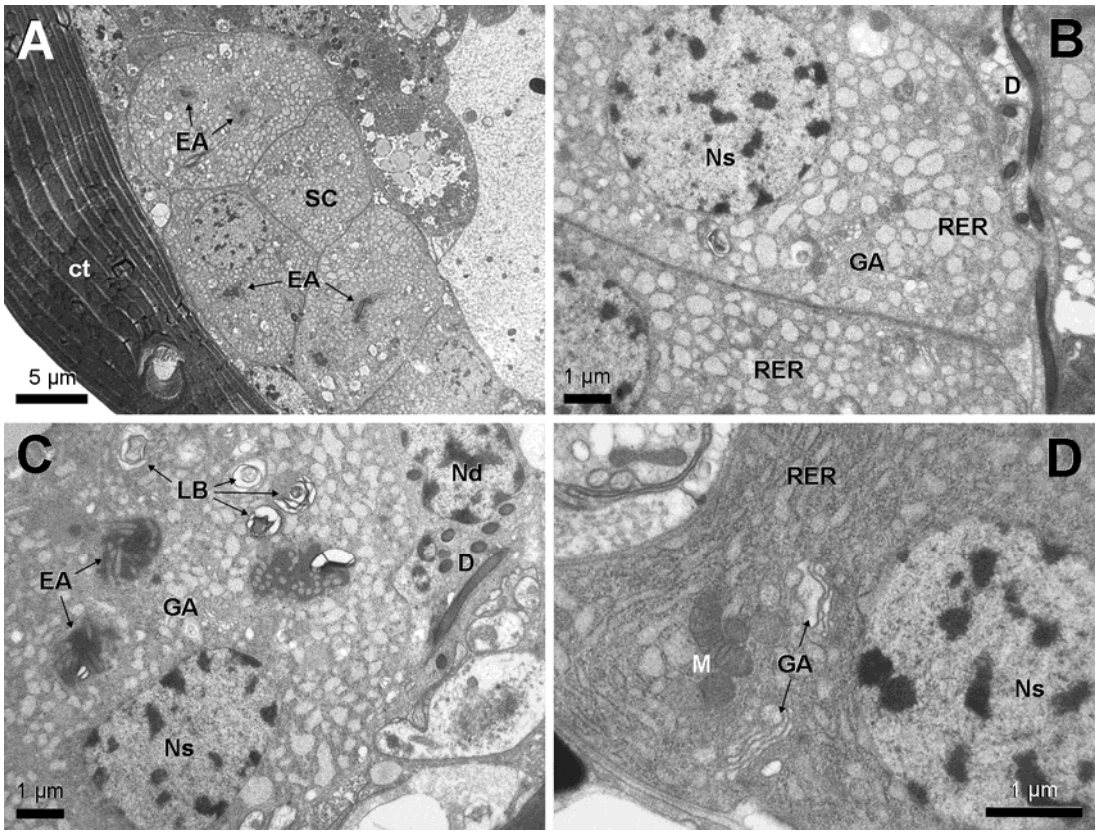

Fig. 2 – Electron micrographs of intramandibular gland cells in minor workers. **A**. Low magnification view of upper secretory cells (SC), illustrating the highly vacuolar cytoplasm. **B**. Lower secretory cells and adjoining ducts (D), showing well-developed granular endoplasmic reticulum (RER) and Golgi apparatus (GA). **C**. Detail of upper secretory cells with sections through end apparatus (EA) and lamellar bodies (LB). **D**. Cytoplasmic detail of upper secretory cell with granular endoplasmic reticulum (RER), Golgi apparatus (GA) and mitochondria (M). ct: cuticle, Nd: nucleus duct cell, Ns: nucleus secretory cell.

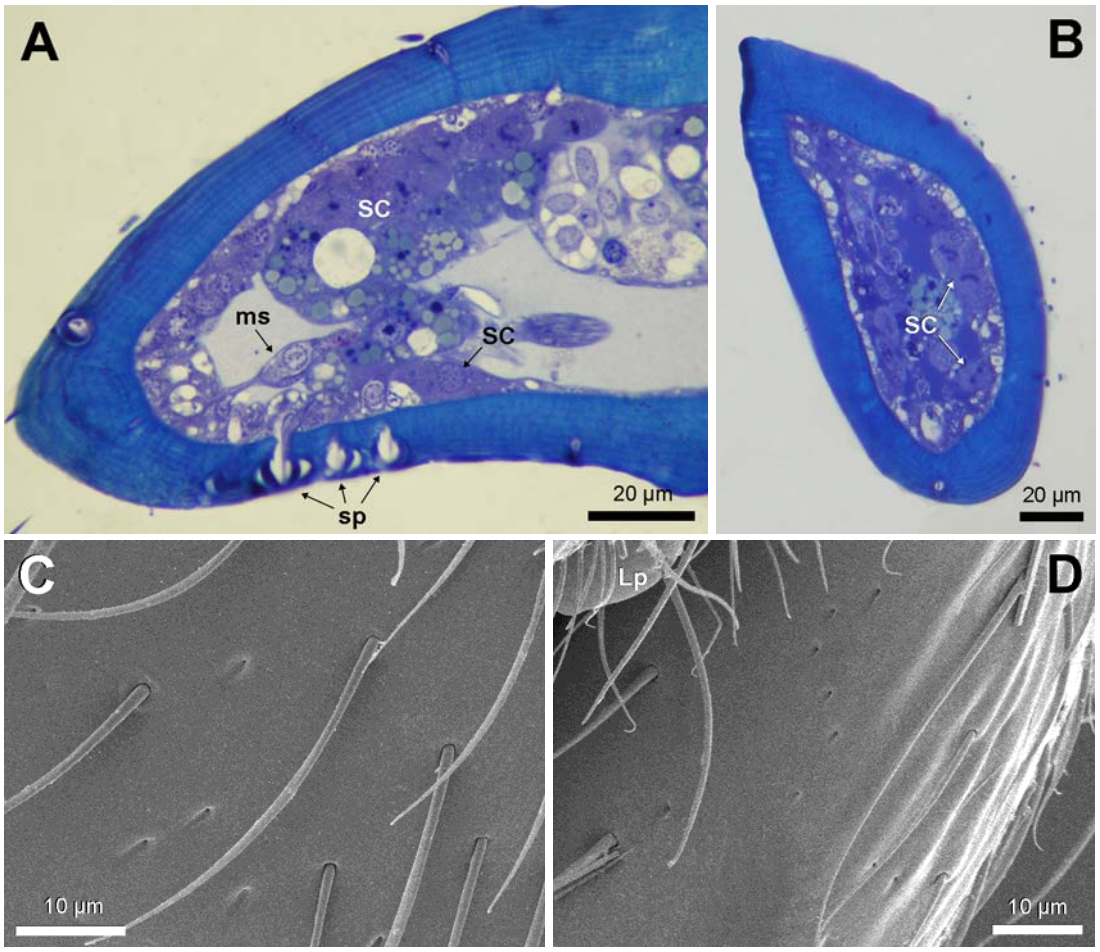
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Fig. 1

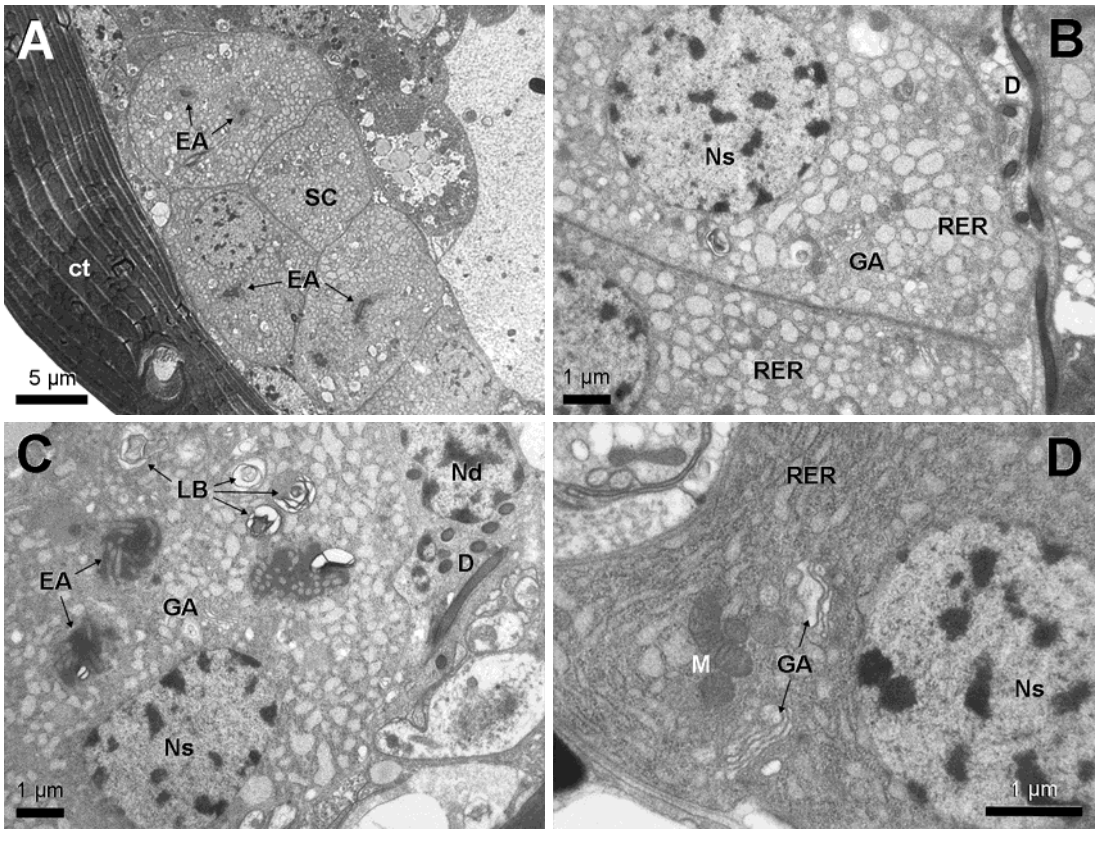
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Fig. 2